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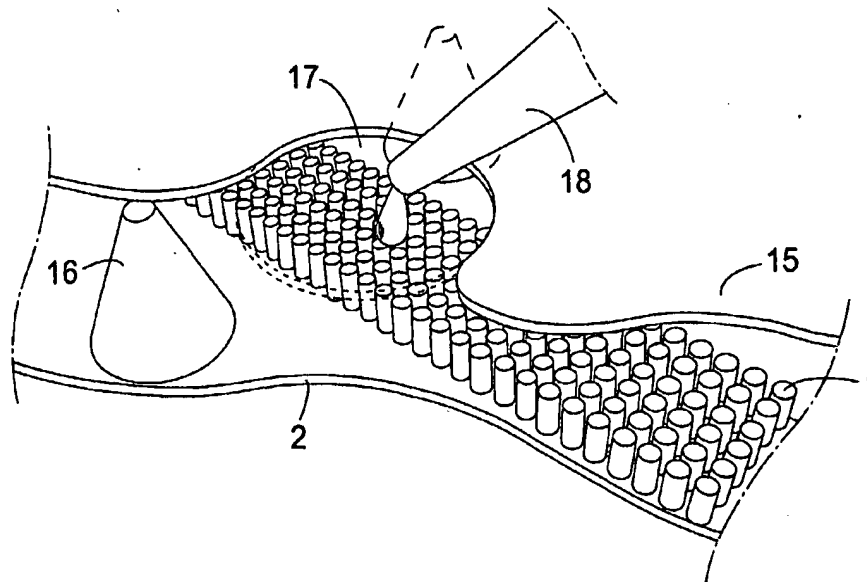
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(54) Title: MICRO FLUIDIC STRUCTURES



(57) Abstract: The invention relates to a micro fluidic system comprising a substrate, and, provided on said substrate, at least one flow path interconnecting with functional means in which liquid samples can be treated by desired procedures. The flow paths are laid out to form a pattern for the transport of liquid samples to and from said functional means. These flow paths comprise a plurality of micro posts protruding upwards from said substrate, the spacing between the micro posts being small enough to induce a capillary action in a liquid sample applied anywhere within any of said flow paths, so as to force said liquid to move from where said liquid sample was applied.

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MICRO FLUIDIC STRUCTURES

Field of the invention

The present invention relates to micro fluidic structures, and in particular to a micro structure defining a liquid flow system, wherein capillary action is utilized as the main driving force for liquid transport through said structure.

The micro fluidic structure according to the invention is useful in various fields of application such as miniaturized bioassays, preparatory steps for such assays, separation, electrophoresis, capillary chromatography, micro reaction cavity procedures, miniaturized liquid communication units, biosensor flow cells and the like.

10 Background of the Invention

Liquid transport through channels or structures on a micro scale has important implications in a number of different technologies.

Controlled transport of fluids through micro channels has been a challenge, with the microstructure itself imparting difficulties not found on a larger scale. The driving force utilized in most micro channel structures depends on electro-endosmosis, gravitational forces, external pressure or capillary migration.

Surface materials often have unbound electrons, polar moieties, or other features generating a surface charge or reactivity. Surface characteristics often have a more pronounced impact on a micro scale system than on a larger structure. This is particularly true in micro systems where the fluid flow is driven by attractions between liquids and the surface materials through which they are transported.

In a closed capillary the driving force is usually represented by the equation:

$$h = 2\sigma_{gl} \cos(\theta_c) / g \rho \quad (I)$$

where h = the height of a fluid within a capillary tube; θ_c = the contact angle of the fluid with the capillary tube material.

If the contact angle of the capillary tube material, with respect to the fluid, is less than 90° , the material is considered hydrophilic. If the contact angle of the tube material, with respect to

the fluid, is greater than 90° , the material is considered hydrophobic. σ_{gl} represents the surface tension of the fluid with respect to the air (millijoules/m²), g is the gravitational constant (m/s²), r is the radius of the capillary tube (m), and ρ is the fluid density (kg/n³).

Planar micro structures have been developed in which a number of grooves or channels are made, typically such a planar structure is produced by etching grooves in a semiconductor
5 substrate, such as a silicon wafer, and then covering the etched surface by a cover plate to complete the channels. Such structures are, however, rather time consuming and expensive to produce.

Further, when such structures need to be customized, e.g. by the addition of chemical reagents,
10 the functionalization of surfaces etc., these steps often need to be performed by somebody other than the producer of the micro structure. In practice, the micro structures are manufactured at one location and shipped to another facility e.g. for the addition of reagents, whereupon they often need to be returned to the manufacturer for closing and sealing. One aim of the present invention is therefore to make available a micro structure offering greater flexibility and ease, in particular
15 with regard to the post-production customization.

Systems used at present utilize external means, e.g. gravity, centrifugal force (spinning of disk elements with micro channels on the surface), or pressure to impose transport of liquids in channels. Also electric fields can be used to impose transport of dissolved charged species in micro systems. To this end, external auxiliary equipment is employed, such as a motor to generate the
20 spinning of a disk, pumps to create pressure, electrodes and power supplies to apply electric fields etc. Such equipment is costly and sometimes rather complex. Furthermore, in certain cases the forces involved in the above mentioned methods could have detrimental effects on sensitive substances. Another aim of the invention is therefore to make available a micro structure with built-in functionality, removing or reducing the need of external means to
25 impose liquid transport.

Prior art

EP 1 120 164 describes the use of a plurality of micro structures in a capillary pathway, said pathway having at least one curved portion, the pathway comprising a base, an inner wall defined by a first radius from a center point and defined by a second radius greater than the
30 first radius, the inner wall and outer wall being fixed to the base and defining the lateral boundaries of the capillary pathway, and a lid extending at least from the inner wall to the

outer wall covering the capillary pathway. It becomes clear that the micro structures themselves do not form a capillary pathway, but only influence the flow as the fluid travels around curved portions in said capillary pathway, the flow being somewhat slower near the inner wall of a curved portion than near the outer wall.

5 U.S. 5,885,527, cited in the above EP 1 120 164 describes assay devices including micro structures forming reaction barriers. These reaction barriers are formed by corrugated or otherwise patterned surfaces, having grooves which together with a cover or top member form narrow channels between different chambers in said device. It is clear from the description and figures, that the capillary channels are indeed formed only when a top member is placed
10 on a bottom member a capillary distance apart. Further, the top and bottom members may be married, the various chambers sealed and the capillaries formed by a number of techniques, including but not limited to, gluing, welding by ultrasound, riveting and the like.

U.S. 5,837,115 discloses a sorting apparatus and method for fractionating and simultaneously viewing individual micro structures, such as cells, viruses, macro molecules
15 or minute particles in a fluid medium. The aim of the invention is to replace agarose gels and other traditionally used fractionation media with a lattice structure with uniform distribution, size and shape of the hindered environment. The hindered environment may be formed by posts, bunkers, v-shaped or cup-shaped structures, forming sifting means for the cells, viruses etc under study. This structure is covered by ceiling means positioned over the
20 lattice causing the migration of microstructures in essentially a single layer through the sifting means exclusively. The invention according to U.S. 5,837,115 does not seem to consider capillary forces, but instead suggests the provision of electrodes for generating an electric field over the lattice structure.

It is widely known to use channels filled with porous material, as exemplified in U.S.
25 5,540,888, said porous material having sub-channels divided by slots, liquid impermeable separating portions etc., and containing reagent regions and sample application regions. Filter paper is often the material of choice, and there exists a wide range of technologies for applying reagents to this material.

It remains to make available micro fluidic structures integrated in a support which is suitable
30 for mass production, and in a configuration which makes the micro fluidic device, comprising said structures, easily handled in the down-stream production process, and in

particular in the customization of the device.

In view of the drawbacks of having to use relatively complex external equipment for influencing liquid flow, which in addition could damage the samples, it would be both desirable and advantageous to enable "automatic" transport of sensitive substances without use of
5 complex devices and without subjecting the substances to the risk of being altered, e.g. denaturated or otherwise damaged.

One aim of the present invention is therefore to provide a micro-fluidic structure, i.e. a geometric micro structure defining a liquid flow system, suitable for capillary transport of liquids and which is inexpensive to produce, optionally permitting a disposable type product, optionally
10 having branched flow channels, optionally exhibiting local surface characteristics, and providing great freedom in choice of material, e.g. with regard to surface, optical and electric properties.

Further aims underlying the invention, as well as the advantages associated with the inventive solution and its embodiments, will become apparent from the following
15 description and examples, together with the attached claims and drawings.

Summary of the Invention

The above aims are achieved with the present invention by employing the phenomenon of capillary action to drive liquid flow through open micro structures provided on solid substrates. In its widest scope, the micro fluidic structure according to the present invention comprises
20 various forms of geometric micro structures defining the desired liquid flow system.

The present invention in its broadest aspect provides a flow path consisting of a multitude of micro structures inducing and/or facilitating the flow of fluids through said flow path, as well as methods where this flow path is used.

The invention in particular provides a micro fluidic system comprising a substrate, and,
25 provided on said substrate, at least one flow path and functional means in which liquid samples can be treated by desired procedures, wherein said at least one flow path is/are laid out to form a pattern for the transport of liquid samples to, through and from said functional means; wherein the flow path consists of a plurality of micro posts protruding upwards from said substrate; wherein the spacing between the micro posts is such as to induce a
30 capillary action in a liquid sample applied anywhere to said flow path, so as to force said liquid to

move from where said liquid sample was applied.

Brief Description of the Drawings

The present invention will be described in closer detail in the following description and non-limiting examples, with reference to the attached claims and drawings, in which:

- 5 Figure 1 is a SEM microphotograph of a portion of a structure according to the invention;
- Figures 2 through 6 show cross sections of the capillary structure according different embodiments of the present invention;
- Figure 7 shows a perspective view of one embodiment of a flow path where the capillary structure is formed of micro posts having a circular cross section; with no walls nor cover
10 which would significantly contribute to the capillary action, according to the present invention;
- Figure 8 illustrates one embodiment of a flow path according to the present invention comprising zones with capillary structures or micro posts having different cross sections and different dimensions in adjacent zones;
- Figure 9 shows schematically in cross section perpendicular to the plane of the device, the use of a
15 bibulous material for maintaining or enhancing flow in a flow path according to the present invention;
- Figure 10 shows schematically how a flow path according to the present invention can be divided into zones, separated by "barriers" preventing the capillary flow, and means for resuming or re-starting the flow;
- 20 Figure 11 shows schematically a cross section of a device having micro structures forming a flow path on a surface, and a separate lid, not significantly contributing to the capillary flow.
- Figure 12 shows schematically a partial exploded view of a device according to the invention, and as illustrated in Fig. 11, where a drop of liquid, e.g. a sample, is being added with a pipette through a hole in said separate lid; and
- 25 Figure 13 shows schematically an embodiment where micro post of different size and/or functionality form a discontinuous gradient.

Description of Preferred Embodiments of the Invention

Before the present invention is described, it is to be understood that the terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

The following terms will be used:

The prefix "micro" as in micro fluidic, micro structure etc is used to define a device or a process which comprises or involves at least one feature having a length, width or height normally expressed in micrometers (μm , $1 \times 10^{-6} \text{ m}$).

10 The prefix "nano" is here used in its generally accepted meaning, as in nanometer (nm, $1 \times 10^{-9} \text{ m}$).

The expression "passive" as used in e.g. "passive control" or "passive fluid dynamics" refers for the purposes of this invention to a control that is not influenced by actions taken during a process that is to be carried out, but rather the control is determined by fixed system parameters, which are design dependent. The passive control is generated by using the natural capillary forces that exist on a micro scale.

The term "open" in this context means that the flow paths defined by the micro structures are accessible from above, and have no cover or lid which takes part in creating the capillary flow. The above definition does however not rule out that a secondary cover or lid, at a distance from the micro structure, can be provided.

The term "hydrophilic groups" refers to substrates and substances having polar and/or charged groups, such as hydroxyl, carboxyl, amino, sulphonate, thiol, aldehyde etc.

The term "hydrophobic structure" refers to substrates and substances having non polar structures.

The term "chemically reactive groups" refers to all organic and inorganic groups used in covalent coupling of molecules to solid faces and known to persons skilled in the art, such a hydroxyl, carboxyl, amino, sulphonate, thiol, aldehyde etc.

The term "biological affinity" refers to substances having specific binding of a substance or a

defined group of related substances. Exemplary substances are antibodies, antigens, haptens, biotin, avidin, lectin, sugar, nucleic acids, hormones and their receptors.

The invention in its broadest aspect provides a multi-element capillary structure adapted to facilitate and/or effect the capillary flow of liquids along said structure in open systems by using
5 micro-structures provided on or in said structure. Advantage is taken of the surface effects between a fluid and the surfaces contacting the fluid. These surface effects come into play at the micro scale.

In particular there is provided devices having a structure comprising at least one liquid flow path, optionally connecting different processing compartments within said structure for
10 carrying out a number of different unit operations. Examples of such processing compartments, elements and/or devices are chemical reaction compartments, incubation compartments, wash compartments or elements, flow control elements, measurement elements, time gates, separation means, heating means, means for irradiation with electromagnetic radiation, magnetic means for trapping magnetic components of said liquid within said
15 functional means, electrodes for applying voltage to the liquid over a selected region, detectors for detecting physical or chemical properties e.g. temperature, pH, viscosity, absorbance etc., or any other device or means for chemically, biologically or physically treating a liquid sample or reaction mixture located within or passing through said compartment, element and/or device.

In order to clarify the principle behind the inventive concept the following illustration is given:

20 Consider a simple example of the effect of surface forces, such as demonstrated when water is drawn into an glass capillary without any outside pressure being applied. This is caused by the surface tension between the water and the glass surface, which pulls water into the capillary. The narrower the capillary, the greater the effect of the force that pulls the water into the capillary. This is often referred to as the capillary force.

25 One physical parameter that characterizes the magnitude of the capillary force is the contact angle between the water and the surrounding material. For contact angles less than 90° , the material, e.g., glass, is considered to be hydrophilic and water is drawn up into the capillary space. When the material has a contact angle greater than 90° it is considered hydrophobic. In the hydrophobic case, pressure is required to force water into the space. The narrower the capillary, the greater the force
30 that is required. However, in both cases once water has been introduced into the capillary, the flow rates of the water depend more on pressure gradients and friction and less on whether the material

is hydrophobic or hydrophilic.

In order to achieve the desired function of the micro structures forming the flow paths of the present invention, the contact area between liquid and the surface of the solid material is maximized, whereby the capillary force increases so that a fluid flow within or along the flow path according to the invention is spontaneously induced and maintained over a desired period of time.

This process represents what can be referred to as "passive fluid dynamics". The present inventors have found that it is advantageous to use such passive fluid dynamics to control and drive the flow of fluid in open micro channels or structures on a surface. For example, the passive nature of the transport mechanism according to the invention makes it direction independent, as compared to a system on a spinning disk where mainly a radial transport is possible. Transport by application of an electric field is mainly bi-directional at best. Capillary flow can be induced in any direction, provided the pattern of micro structures forming the flow paths is designed properly. A person skilled in the field of designing micro structures and fluid flow cannerls on such structures will be able to apply the teaching of the invention without undue experimentation.

Figure 1 shows a SEM microphotograph of an example of a micro fluidic structure embodying the inventive concept. It is evident from the picture that the micro posts have an identical shaped, a regular form and are evenly spaced over the support structure. Also the surface between the micro posts is even. A skilled person will recognize that the micro posts shown in the SEM microphotograph have a high aspect ratio. In this example, the micro posts were about 100 μm high, had a diameter of 20 μm and a center-to-center distance of 30 μm . This accounts for an aspect ratio of 1:5. It is generally held that an aspect ration > 1:2 is a high aspect ratio.

Figures 2 through 6 show a number of different cross sections of the micro structures forming the fluid flow path according to the present invention. The micro structures or micro posts can have a cross section which is one of circular, elliptical, rhombic, triangular, square, rectangular, heptagonal, hexagonal etc or a combination thereof. The cross section can also be any fraction of the above forms, such as a half-circle, a crescent, U-shaped, X-shaped etc as long as the dimension and center-to-center distance of the individual microstructures is such, that capillary flow is induced without the provision of any lid or cover, limiting the flow path.

Figure 7 shows schematically a flow path consisting of a multitude of circular micro posts 1 on a surface 2. The micro posts can naturally have any cross section, height and center-to-center distance, as long as the parameters are chosen such that capillary flow is induced. The direction of the capillary flow is indicated by the black arrow. In Fig. 7, the support 2 is schematically indicated as having a thickness comparable to the height of the micro posts. While this is not ruled out, the most frequently encountered supports will be considerably thicker. Figure 7 is thus only a schematic illustration.

A device according to the invention may comprise a support and at least one flow path consisting of micro structures as shown in Figure 7. However, for most practical applications, a multitude of flow paths forming a channel system is required. The individual channels connect different functional regions, means or devices, such as reaction chambers, separation media etc.

When the properties of the columns and their properties, such as their material, their shape and/or distance, and optionally also the properties of the substrate, are selected properly with due consideration taken to the liquid to be transported, it will become possible to create a capillary flow through said structure, in the direction of the arrow if a liquid sample is placed at the end where the arrow points, without any significant leakage out from the structure and onto the surrounding substrate surface.

Thus, the basic structure embodying the inventive concept is a substrate having at least one flow path provided in or on its surface. This flow path or channel is formed by column like micro structures or micro posts, protruding from surface of said support. The characteristic feature of the flow path and the micro posts therein is that the dimensions of said posts and the distance between said posts are selected such that capillary flow of liquids can be maintained therein. In particular, the distance between said columns is in the range of 0.1 - 1000 μm , preferably 1 - 100 μm . The columns are preferably higher than 1 μm , more preferably higher than 10 μm . Most preferably said micro posts have a high aspect ratio, that is a width to height ratio greater than 1:2.

It is understood that the micro posts can be either positioned within a secondary structure on the surface, such as a groove or a depressed area, or directly on the surface, protruding there from. When the micro posts are in a secondary structure, such as a groove in a substrate, the flow path will have a bottom located beneath the general substrate surface, and

more or less vertical side walls, together with the bottom forming a channel. The capillary action inducing and/or maintaining the flow is however caused essentially by the interaction between the liquid and the micro posts.

However, according to a preferred embodiment, the micro posts or columns are laid out as regions, preferably elongated, of upstanding columns, without any delimiting side walls. All functions and features that have been or will be discussed herein with reference to ordinary channels, are equally applicable to this type of structure, which thus is fully within the scope of the inventive concept as defined in the claims.

According to a further embodiment of the present invention, the flow path is subdivided into zones wherein the columns can have different column height, diameter, geometry and/or different column density, i.e. number of micro-posts per unit area. Figure 8 is a schematical illustration of this embodiment. A plurality of micro posts 1 are provided in close proximity in adjacent groups of micro posts having different size, shape or functionality. In Figure 8, these groups are indicated 3, 4, and 5 and the differences shown as different size, shape and spacing only for the sake of illustration. Such groups can form an array, having desired functionality. Preferably said groups form a gradient, which can be continuous or discontinuous, preferably continuous.

In the embodiment shown in Figure 8, a first more dense zone 3 is provided, where the micro structures have a smaller diameter and smaller distance. Such zone can act as a sieve or "fence" preventing larger particles, e.g. cells from passing. Next, there is a zone 4 with posts having relatively large spacing between them. This can serve to temporarily decrease the time for a liquid-solid face interaction in a desired region, e.g. if it is desired that the sample be exposed to some surface bound moiety for an specified time, in order for a particular reaction to proceed to a reasonable completion etc. After this low velocity region there is provided a zone 5 of larger micro posts (squares in the shown example) having fairly narrow passages between them. After this region, a second zone 4 similar to that is provided. Based on the information given in this description and the examples, a skilled person can design various combinations, according to the desired purpose.

According to another embodiment of the invention, the capillary flow path or paths has/have integrated surfaces or zones composed of bibulous material capable of capillary transport. This is schematically illustrated in Fig. 9 which shows a simple application of this concept.

In this case there is a closed channel structure comprising a plurality of micro-posts 1 filling the channel. The structure has a bottom substrate 2 and a cover 6, the substrate also forming side walls (not shown), and having an input aperture or hole 7, an optional further aperture or hole 8, and an exit aperture (not shown). If a drop 9 of liquid is applied to the input
5 aperture 7, a capillary flow will immediately begin, and will continue to draw liquid from the drop until the flow reaches the exit, where no further capillary action will occur. However, if a bibulous material such as pad 10 of filter paper or the like of sufficient size, is applied at, within or in contact with the exit aperture, this material will by its capability of drawing liquid act as a "flow sink", i.e. it will absorb the liquid that exits from the channel, thereby
10 enabling an essentially continuous flow through the channel.

Another embodiment having a flow sink, is that of the closed channel ending in an open region or zone of micro posts, said region having a relatively large area as compared to the channel. This large region will act as a flow sink in the same sense as the bibulous material discussed above. If e.g. heating means is provided to heat this region, evaporation of the liquid can be induced, thereby
15 creating a sink that in principle could be maintained indefinitely. Evaporation of the liquid will also make possible the capture of components present in the flow, at a desired location and at a desired time in the device or process employing such device.

Figure 10 shows schematically another embodiment and application of the flow path structures according to the invention, the flow path comprising a plurality of consecutive micro fluidic
20 structures, interrupted by zones without such structures, i.e. a small space or discontinuity 11 and 12, said discontinuity acting as capillary barriers, preventing liquid from being transported across the zones without assistance. The direction of the liquid flow is illustrated with the large horizontal arrow.

The discontinuities can be bridged or assisted transport can be brought about e.g. by applying a
25 pressure pulse, whereby the capillary barriers provided by the voids are broken. The means for creating the pulse can be implemented by providing a very small channel 13 and 14 opening into the spacing, and for example momentarily applying sub-pressure (indicated with vertical arrows) in said channels. At the other end of the channel some means for providing a slight sub-pressure can be provided, whereby liquid from the regions on each side of the discontinuity will be forced into
30 it, and when the gap is filled, capillary flow will again be resumed. This requires a closed system. In an open system (and in a closed), the small channel can be used to introduce liquid to fill the gap, thereby restoring a flow connection so as to resume the capillary flow.

Figure 11 shows a schematic partial view of a device according to the invention, where micro posts 1 are provided on a substrate 2, said substrate having larger protrusions 16 for carrying a cover or lid 15, said protrusions defining a distance between the surface the substrate and the lid considerably larger than the height of the micro posts, and such that no capillary interaction
5 between the substrate and the lid can arise. The same applies to an alternative embodiment where the micro posts are located in grooves or depressions in the surface. The cover or lid 15 may further have apertures or holes 17, said holes preferably indication points for adding a sample or a reagent, for reading a result or for following the advancement of the reaction/reactions taking place on the substrate.

10 According to a preferred embodiment, the cover or lid is attached to the protrusions 16 only after the surface of the substrate has been functionalized or customized, i.e. after the addition of the necessary reagents and/or functionalities.

Figure 12 shows schematically an exploded view of the embodiment of Figure 11, where a pipette tip 18 is shown deposition of a drop of a liquid through an opening 17 on a flow path
15 according to the invention.

Figure 13 illustrates an embodiment where groups of micro structures within a continuous flow path form a gradient with respect to at least one property, e.g. the shape, size, center-to-center distance or a functional / chemical property. In the figure, the flow path comprises a discontinuous gradient with respect to the size and center-to-center distance of the micro structures, represented
20 by the groups A, B, C and D. A gradient like this can function to delay the passage of biological or chemical entities, such as particles, cells, organelles, macro molecules or the like, in a desired manner, or separate such entities. Entities captured in zone A are illustrated by the shape "a", entities captured in zone B with "b" and so on. The shape "e" illustrates entities that pass unhindered through the gradient.

25 In further embodiments, the flow paths can comprise integrated surfaces or zones containing a number of different functional elements or devices for performing a number of different operations on the media located within or in association to the flow paths. Examples of such functional elements or devices are electrodes and/or other means of electrical manipulation of liquids and reagents. The electrical manipulation can e.g. comprises oxidation/reduction of
30 species. Other representative examples of functional devices are optics and other means to manipulate light e.g. for the purpose of measuring concentrations by absorbance, inducing

conformational changes by light irradiation.

Magnets or means for detection of magnetic substances can also be arranged in or around the flow paths. Thereby, magnetic particles can be trapped and retained at desired locations in the structure, and the magnetic property of the particles can thereby be used as a marker or
5 indicator of successful transport to a certain point in the system. Furthermore, magnetic particles can be coated with substances with biological affinity and used in different kinds of assays.

Furthermore, means for the manipulating of temperature can be provided in selected locations in a flow path according to the invention. Thereby a number of interesting functions can be
10 performed such as, chemical reactions, incubation, thermal curing, PCR reactions, evaporations, drying etc.

Within the flow path structures according to the invention there can be provided particles within or in association to the flow paths, at least in one of the zones containing micro structures such as micro posts or columns, where there is subdivision between zones with and without these
15 structures. The particles can thereby be bound by physical forces to said substrate, or chemically bound, e.g. covalently bound to said substrate. Alternatively, said particles can be mechanically trapped within the zone (zones) containing said structures.

According to a preferred embodiment, the substrate has reactive substances attached to its surface. Such reactive substances are used in connection with detection of substances of
20 chemical or biological origin. The reactive substances are used in connection with immobilisation of substances of biological as well as non-biological origin, i.e. they are provided as sites to which the substances to be immobilized can react so as to become bound to the substrate.

Another application of a reactive substance provided on the substrate is the use thereof in
25 connection with separation of substances of biological as well as non-biological origin, where the substance can be selectively reactive with a species that one wishes to separate from another in a mixture.

The surfaces of a channel in a structure according to the invention can be modified by chemical or physical means. Such a modified surface can thereby be used in connection with
30 detection of substances of biological and chemical origin. A modified surface of this kind can

be used in connection with immobilisation of substances of biological as well as non-biological origin. It can also be used in connection with separation of substances of biological as well as non-biological origin.

5 Still another application of the flow path or channel structure according to the invention, is that the flow paths are used as means for multi spot detection, whereby said paths optionally have particles provided therein, said particles having chemically reactive groups or substances with bio-affinity bound in the micro spot area.

Other suitable applications of flow path structures according to the invention is the measurements of the amount of an analyte in a biological sample, e.g. in blood, serum, plasma,
10 urine, cerebral spinal fluid, tears, amniotic fluid, semen or saliva, the measurements preferably being based on specific biological interactions.

A specific application is that said analyte is measured by immunological means.

The analyte can also be detected by specific interactions using poly- or oligonucleotides, preferably single stranded nucleic acids or aptameres.

15 The flow path structures according to the invention can also be used for the separation of cells, and for the screening of synthetic or biological libraries.

As discussed above, the notion of a "channel" for the purposes of the present invention goes beyond the ordinary concept of a channel, by allowing entirely open structures having no physical delimitations except for a bottom substrate on which the micro post or column
20 structures are provided.

However, when the channel or flow path comprises a groove (i.e. having a bottom and side walls), there are two options: i) to leave the channel open upwards, and ii) to place a top cover so as to make a closed system.

25 These two different embodiments have certain merits for different applications. In cases where it is desirable to manipulate the liquid that is transported and/or the substances transported by said liquid, it may sometimes be more convenient to have unrestricted access from above, such as for the purpose of adding reagents at desired points in the structure, to apply direct heat, or to perform other manipulations at more or less arbitrarily points.

Certain systems may be very sensitive to oxygen, and therefore it may be absolutely necessary to exclude the sample liquid from atmospheric exposure. If the micro-fluidic structure is covered, there can be provided access openings in said cover for enabling introduction of e.g. reagents, gas, liquids, or samples into said structure. Such access openings
5 can also be used for connecting external equipment, e.g. via suitable tubing or the like.

However, both these embodiments are within the inventive concept.

Manufacturing of such microstructures could in its simplest form be done by direct curing of a photosensitive mono- or pre-polymer deposited on a substrate, employing a mask through which light is irradiated to initiate curing, and thereafter rinsing away the un-cured areas (thick film
10 photo-resist process).

An other straightforward method is through replication of an original into a polymer. The original could be manufactured in silicon through a DRIE-process (Deep Reactive Ion Etch) where high aspect ratio structures could be produced. Other ways of producing such originals could for instance be through laser processing, electro discharge methods, Free Form
15 Manufacturing (FFM), electrochemical or chemical etching, gas phase etching, mechanical processing, thick film photoresist processes or combinations thereof, of or on a substrate of, for instance, silicon, glass, quartz, ceramic, metal or plastic material. e.g. PMMA or Teflon.

The most straight forward method of replication would be casting of a mono- or pre-polymer over an original with the desired negative shape. Other ways of producing the polymer replicas could
20 involve injection molding or embossing of thermoplastics or thermoset materials.

If the original in some aspects are not withstanding the replication process a an intermediate replica in a suitable material (a stamper) could first be produced from the original. Examples of such stamper process could be to first deposit a conducting layer on top of the original and thereafter through electroplating form a negative from the original. Certain plating materials
25 such as Nickel lend themselves also to the repeated and non-destructive production of copies of the stamper. This gives the possibility to both change polarity from negative to positive as well as producing series of identical stampers for large volume production of replicas. Other examples of stamper manufacturing could be in a well chosen polymer given the negative shape of the original in a casting, embossing or injection molding process. The same possibility of repeatedly
30 and non-destructively making copies of the stamper could also be true for polymer stampers.

As mentioned above the micro-fluidic structure of the invention may, of course, be designed for a plurality of micro-fluidic purposes. Among those are e.g. capillary chromatography, ion-exchange chromatography, hydrophobic interaction chromatography, immunoassays, hybridization assays and other molecular biology assays, micro reaction cavity procedures, miniaturized liquid communication units, biosensor flow cells, etc. Reaction cavities constructed in accordance with the invention may, for example, be used for various forms of solid phase synthesis, such as peptide or oligonucleotide synthesis. PCR, DNA solid phase sequencing reactions, sample treatment and detection, just to mention a few.

The micro fluidic structures according to the invention can be made in different ways. One convenient method is outlined above, but it is also possible to make the structures from separate parts which are assembled after column formation has taken place in a suitable substrate.

In the following, the invention will be illustrated by specific non-limiting examples.

Examples

Example 1. Flow in open channels with columns made of silicon

Flow channels were produced by etching silicon wafers by a standard method well known to a person skilled in the art. The resulting silicon chips had a length of 25 mm and a width of 5 mm.

- 5 The area covered by columns was 10 mm long and 4 mm wide. The columns had a height of 100 μm and a diameter of 20 μm , the center-to-center distance being 30 μm .

Capillary flow was tested with purified water, buffer and blood plasma. A wicking membrane (Whatman WF 1.5) was placed a few mm in at the distal end of the chip to facilitate the liquid flow.

- 10 8 μl of water added to the structure took between 60 and 90 seconds to flow across the structure provided with columns. A similar flow speed was measured with a buffer containing 50 mmole/l sodium phosphate, 6 % bovine serum albumin, 0,2 % Tween 20, pH 7.5. Blood plasma was slightly faster than water and buffer.

Example 2. Flow in open channels with columns made of epoxy plastic

- 15 Flow channels were produced by first etching silicon wafers by a standard method well known to a person skilled in the art. A thin layer of epoxy was the applied uniformly to the silicon wafer. The resulting epoxy covered chips had a length of 25 mm and a width of 5 mm. The area covered by columns was 10 mm long and 4 mm wide. The columns had a height of approximately 90 μm and a diameter of approximately 20 μm , the center-to-center distance being close to 30 μm .

- 20 Capillary flow was tested with purified water and buffer. A wicking membrane (Whatman WF 1.5) was placed a few mm in at the distal end of the chip to facilitate the liquid flow. The chip was pretreated (one hour, room temperature) with a buffer containing 50 mmole/l sodium phosphate, 6% bovine serum albumin, 0,2% Tween 20, pH 7.5 prior to addition of water or buffer. 8 μl of water added to zone 1 took about 90 seconds to flow through the zone with
25 columns. Similar flow speed was found with a buffer containing 50 mmole/l sodium phosphate, 6% bovine serum albumin, 0,2% Tween 20, pH 7.5.

The above examples demonstrate that it is possible to create open flow paths consisting of micro structures and that these function, i.e. that the necessary capillary forces are created and that liquid transport takes place.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in
5 the claims appended hereto.

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Claims

1. A micro fluidic system comprising a substrate (2), and, provided on said substrate, at least one flow path and functional means in which liquid samples can be treated by desired procedures, wherein said at least one flow path is/are laid out to form a pattern
5 for the transport of liquid samples to, through and from said functional means;
characterized in that the flow path consists of a plurality of micro posts (1) protruding upwards from said substrate; wherein the spacing between the micro posts is such as to induce a capillary action in a liquid sample applied anywhere to said flow path, so as to force said liquid to move from where said liquid sample was applied.
- 10 2. The micro fluidic system according to claim 1, wherein the functional means (5) comprise one or more of chemical reactors, separation means, heating means, means for irradiation with electromagnetic radiation, magnetic means for trapping magnetic components of said liquid within said functional means, electrodes for applying voltage to the liquid over a selected region, or any other device or means for chemically, biologically or physically
15 treating liquid samples located within said functional means.
3. The micro fluidic system according to claim 1 or 2, wherein said substrate (2) is provided with grooves having a bottom surface and side walls, and wherein the flow paths consist of micro posts (1) protruding from the bottom surface of said grooves.
4. The micro fluidic system according to claim 3, wherein said flow paths are covered by a
20 top or lid (15), said top or lid not significantly contributing to the capillary action in said flow channel.
5. The micro fluidic system according to claim 4, wherein the lid (15) has access openings for enabling introduction of reagents, gas, liquids, samples into said flow paths.
6. The micro fluidic system according to claim 1 or 2, wherein the substrate (2) is essentially
25 flat and the micro posts (1) protrude from said substrate.
7. The micro-fluidic system according to any one of the preceding claims, wherein the micro posts are grouped in adjacent segments so as to provide a spacing or discontinuity between such segments, the discontinuity having a finite distance, which is large enough to prevent capillary flow between said segments, thereby providing a flow stop.

8. The micro fluidic system according to claim 7, wherein there are provided means for applying energy to one or more selected segments so as to induce a forced transport across the flow stop.
9. The micro fluidic system according to claim 8, wherein said energy applying means is
5 selected from a pressure pulse generator, an ultrasound generator, an electromagnetic radiation means.
10. The micro fluidic system according to claim 7, comprising means for applying liquid to said discontinuity in order to provide a bridge across said discontinuity so as to induce a flow there across.
- 10 11. The micro fluidic system according to claim 1, wherein the surfaces of the micro posts in said fluid paths have a chemical, biologic or physical functionality.
12. The micro fluidic system according to claim 11, wherein the micro posts have chemically reactive groups on their surface.
13. The micro fluidic system according to claim 11, wherein the micro posts have
15 substances with biological affinity bound to their surface.
14. The micro fluidic system according to claim 11, wherein the micro posts have hydrophilic groups on their surface.
15. The micro fluidic system according to claim 11, wherein the micro posts have positively and/or negatively charged groups on their surface.
- 20 16. The micro fluidic system according to claim 12, wherein the micro posts have hydrophobic structures on their surface.
17. The micro fluidic system according to claim 12, wherein said functionality is applied to the micro posts in the entire flow paths or limited to a discrete region or portion of the flow path.
- 25 18. The micro fluidic system according to claim 12, wherein said properties are selected from the micro post diameter, height, shape, cross section, surface coating, number of micro-posts per unit area, wetting behavior of the micro-post surface, or a combination thereof.

19. The micro fluidic system according to any one of the preceding claims, wherein particles are provided within said at least one flow path.
20. The micro fluidic system according to claim 19, wherein said particles are chemically or physically bound to the substrate, or mechanically trapped within a region comprising a plurality of micro posts.
21. The micro fluidic system according to any one of the preceding claims, wherein said flow paths have integrated zones or delimited surfaces containing electrodes or other means for electrical manipulation of liquids and/or reagents.
22. The micro fluidic system according to any one of the preceding claims, wherein said flow paths have integrated zones or delimited surfaces containing optical elements or other means for transmitting, focusing, reflecting or absorbing light.
23. The micro fluidic system according to any one of the preceding claims, wherein said flow paths have integrated zones or delimited surfaces containing magnetic functionalities or means for the manipulation and/or detection of magnetic substances.
24. The micro fluidic system according to any one of the preceding claims, wherein said flow paths have integrated zones or delimited surfaces containing means for the regulation of the temperature in said zone, e.g. heating or cooling said zone.
25. A method for performing micro scale chemical or biochemical processes, **characterized** in that micro fluidic system according to claim 1 is used.
- 26 A method for performing ion-exchange chromatography, **characterized** in that a micro fluidic system according to any one of claim 1, 2, 11, 15, or 19, is used in the separation step for said chromatography.
27. A method for performing hydrophobic interaction chromatography, **characterized** in that a micro fluidic system according to claim 1 is used in the preparatory steps for said chromatography.
28. A method for the concentration of samples in the performance of micro scale physical, chemical or biochemical processes, **characterized** in that a micro fluidic system according to claim 1 is used.

29. A method for the separation of cells or other microscopic biological entities in the performance of micro scale physical, chemical or biochemical processes, characterized in that a micro fluidic system according to claim 1 is used.

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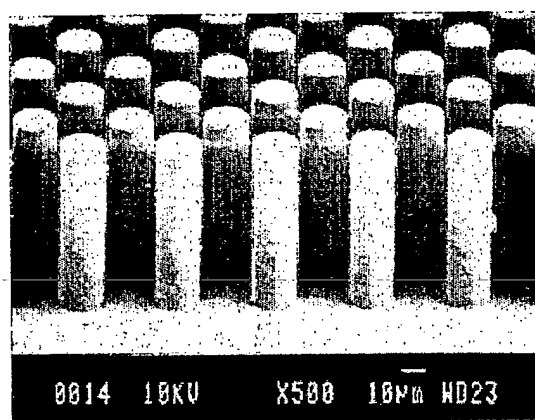


Fig. 1

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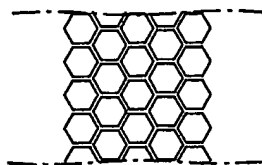


Fig. 2

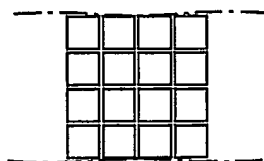


Fig. 3

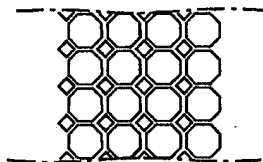


Fig. 4

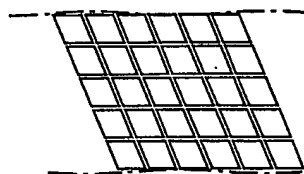


Fig. 5

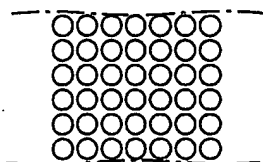
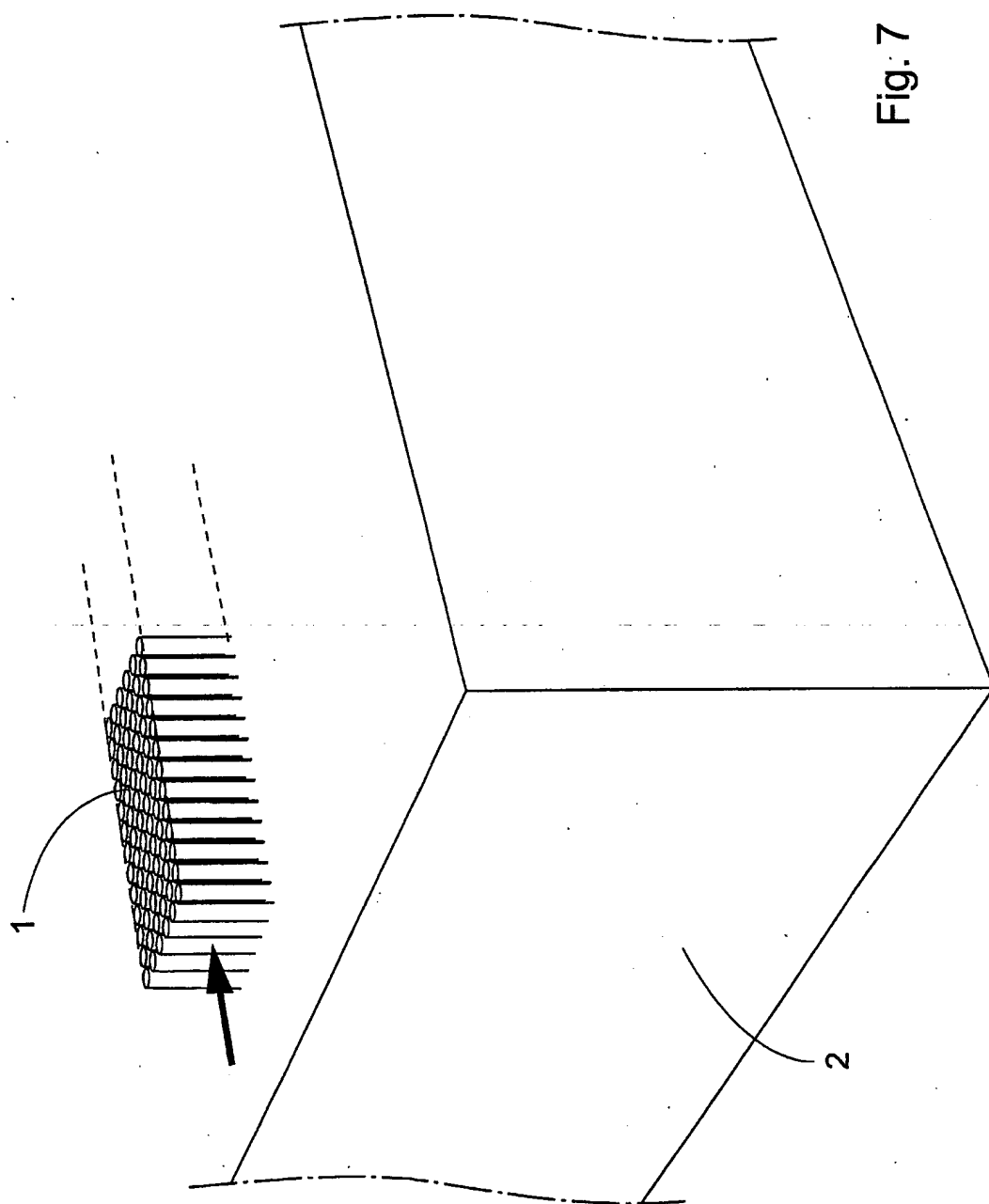


Fig. 6

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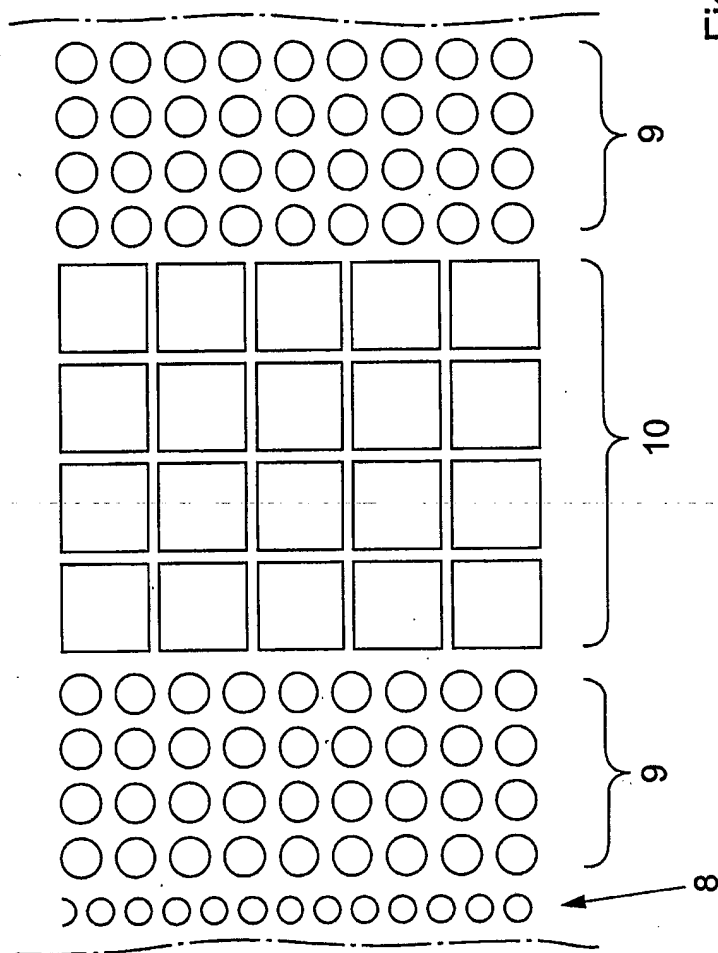


Fig. 8

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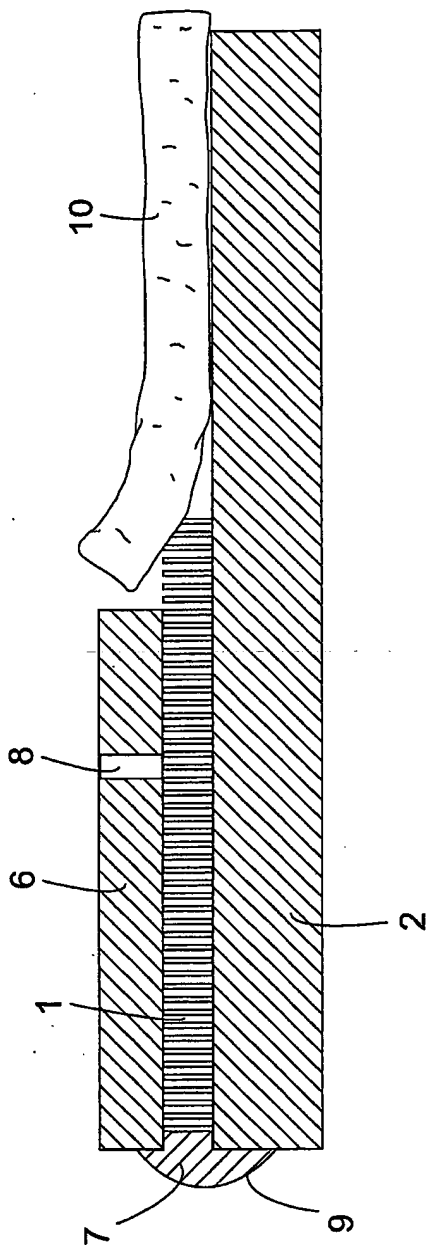


Fig. 9

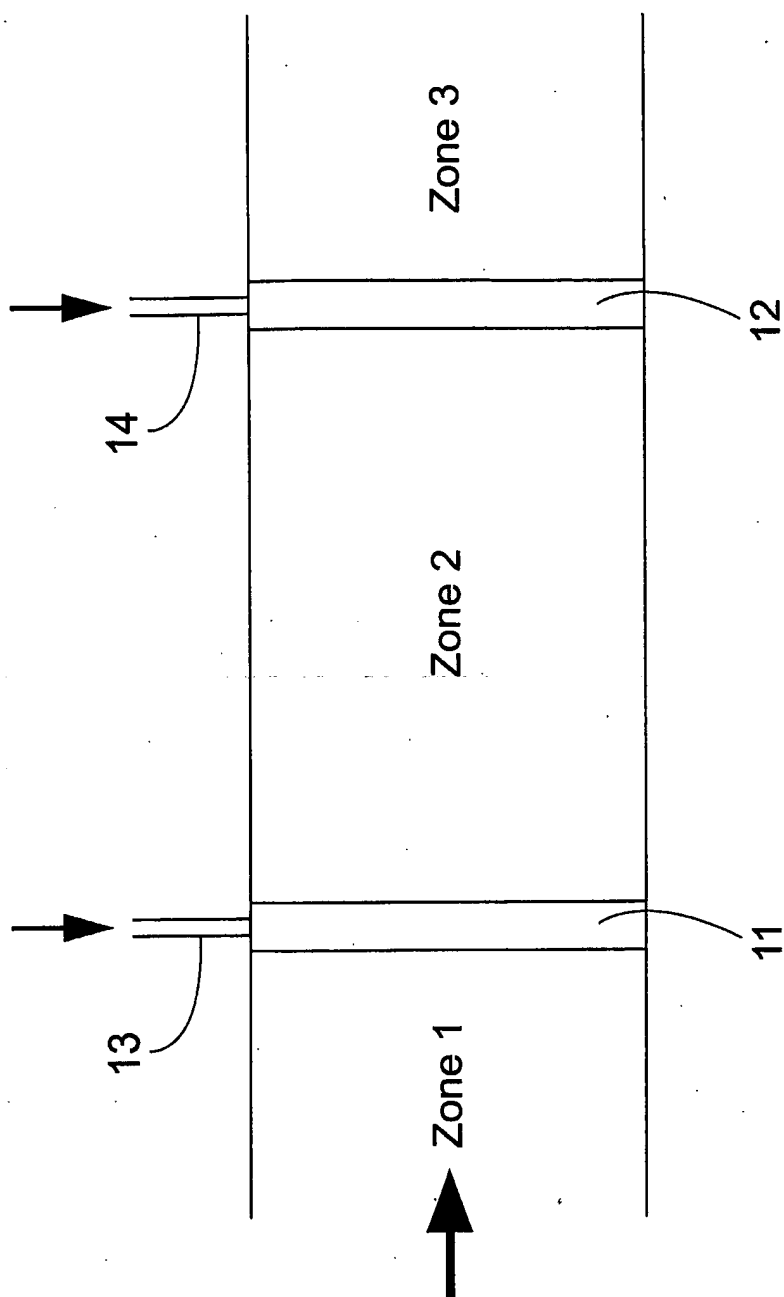


Fig. 10

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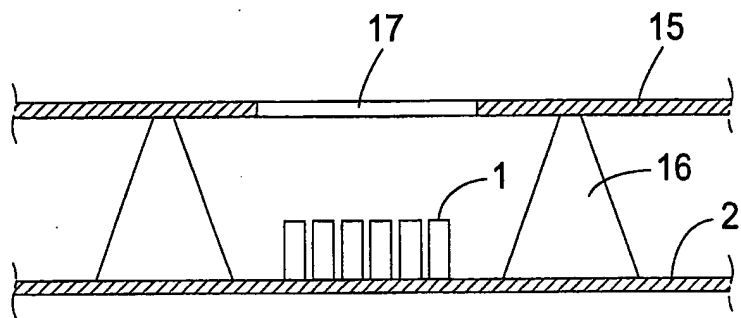


Fig. 11

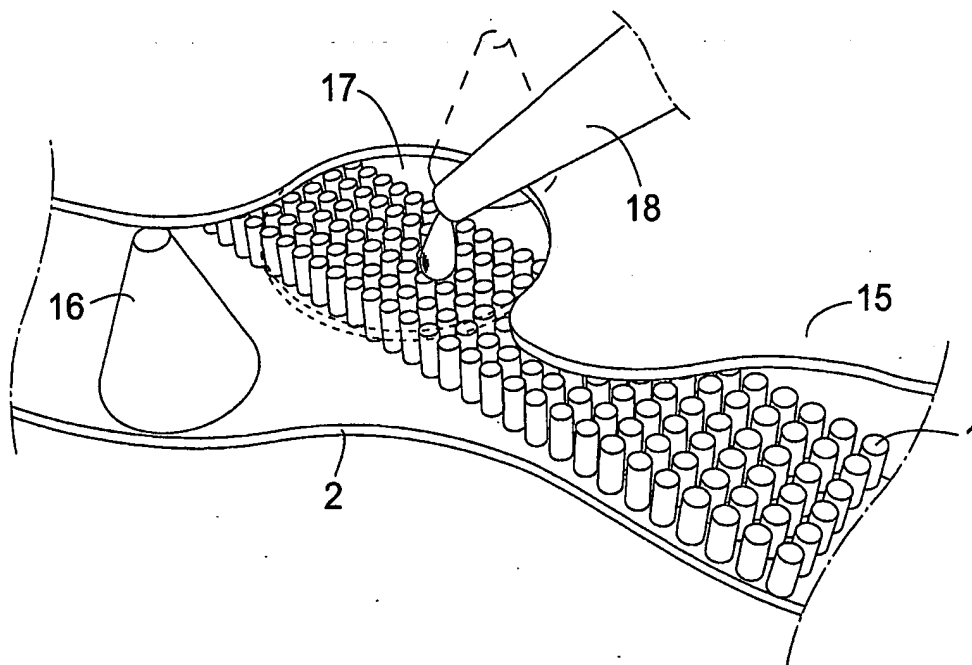


Fig. 12

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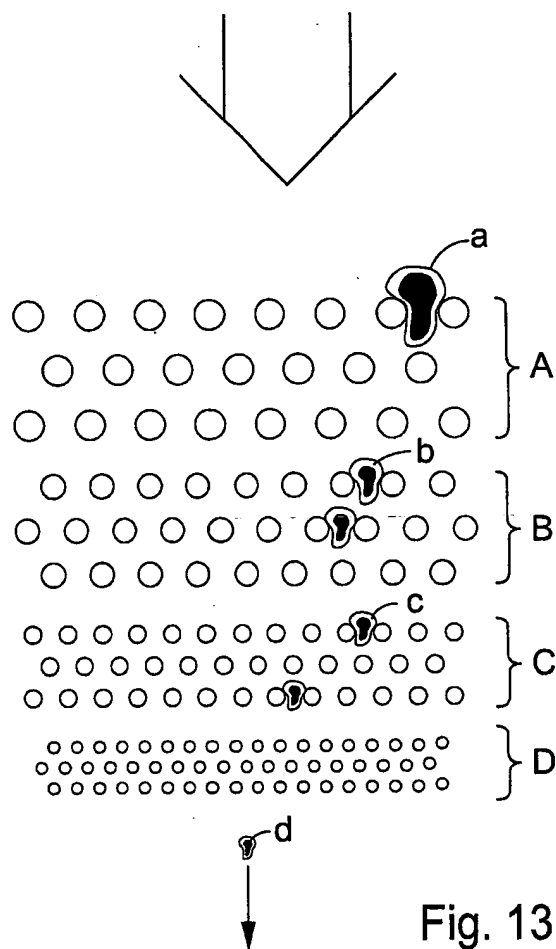


Fig. 13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 03/00919

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: B01L 3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: B01L, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1120164 A2 (ROCHE DIAGNOSTICS GMBH), 1 August 2001 (01.08.01), whole document	1-7, 11-22, 24-29
Y	--	8-10, 23
Y	US 5837115 A (R.H.AUSTIN ET AL), 17 November 1998 (17.11.98), abstract	8-10, 23
A	US 6156273 A (F.E.REGNIER ET AL), 5 December 2000 (05.12.00), abstract	1-29
A	US 5540888 A (R.A.BUNCE ET AL), 30 July 1996 (30.07.96), whole document	1-29

☐ Further documents are listed in the continuation of Box C.
 ☒ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

14 August 2003

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

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Patent document cited in search report			Publication date	Patent family member(s)		Publication date
EP	1120164	A2	01/08/01	CA	2331588 A	28/07/01
				US	6451264 B	17/09/02
US	5837115	A	17/11/98	CA	2164720 A	22/12/94
				EP	0711412 A	15/05/96
				JP	9504362 T	28/04/97
				US	5427663 A	27/06/95
				WO	9429707 A	22/12/94
US	6156273	A	05/12/00	EP	0985146 A	15/03/00
				WO	9854568 A	03/12/98
US	5540888	A	30/07/96	AT	156272 T	15/08/97
				DE	69221320 D,T	20/11/97
				EP	0613559 A,B	07/09/94
				SE	0613559 T3	
				GB	2261283 A,B	12/05/93
				GB	9123903 D	00/00/00
				GB	9223546 D	00/00/00
				JP	7501148 T	02/02/95
				WO	9310457 A	27/05/93
				US	5354538 A	11/10/94